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Bryan F. Singer*, Myranda A. Bryan, Pavlo Popov, Terry E. Robinson, Brandon J. Aragona

Biopsychology Area, Department of Psychology, University of Michigan, Ann Arbor, Michigan, USA, 48109

* Corresponding Author:
Dr. Bryan F. Singer
Department of Psychology
University of Michigan
530 Church Street, East Hall
Ann Arbor, MI 48109.
E-mail: bfsinger@umich.edu

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Abstract

Repeated intermittent exposure to cocaine results in the neurochemical sensitization of dopamine (DA) transmission within the nucleus accumbens (NAc). Indeed, the excitability of DA neurons in the ventral tegmental area (VTA) is enhanced within hours of initial psychostimulant exposure. However, it is not known if this is accompanied by a comparably rapid change in the ability of cocaine to increase extracellular DA concentrations in the ventral striatum. To address this question we used fast-scan cyclic voltammetry (FSCV) in awake-behaving rats to measure DA responses in the NAc shell following an initial intravenous cocaine injection, and then again 2-hours later. Both injections quickly elevated DA levels in the NAc shell, but the second cocaine infusion produced a greater effect than the first, indicating sensitization. This suggests that a single injection of cocaine induces sensitization-related plasticity very rapidly within the mesolimbic DA system.

Highlights

- A single cocaine injection rapidly induces DA sensitization in the NAc shell
- The time-course of NAc DA sensitization corresponds with reports of VTA plasticity
- Individual variation exists in the degree of DA sensitization

Keywords

Cocaine, Sensitization, Dopamine, Voltammetry, Nucleus Accumbens, Psychostimulant

Abbreviations

Dopamine (DA), Nucleus Accumbens (NAc), Ventral Tegmental Area (VTA), Long-Term Potentiation (LTP), Fast-Scan Cyclic Voltammetry (FSCV)

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Declaration of Conflicting Interests

There are no perceived conflicts of interest to report.

Manuscript

Exposure to psychostimulant drugs enhances their later ability to increase the extracellular concentration of dopamine ([DA]) in the nucleus accumbens (NAc), as well as facilitate the acquisition of drug self-administration and motivation for cocaine [1,2]. Indeed, a single psychostimulant injection is sufficient to induce both neurochemical and behavioral sensitization [3,4], suggesting that this form of drug experience-dependent plasticity can occur quickly, and may thus increase the probability of continued drug use. It is thought that the initial neuroadaptations responsible for the induction of sensitization take place on the cell bodies of DA neurons in the ventral tegmental area (VTA), but its later expression involves, in part, changes in DA release at DA terminal fields in the ventral striatum [2].

Within 3-hours after initial exposure to cocaine, the excitability of neurons in the VTA is enhanced via the induction of long-term potentiation (LTP) and accompanying increases in AMPA/NMDA receptor ratios [5]. Although a single injection of cocaine [6] or amphetamine [7] is sufficient to increase the extracellular [DA] in the dorsal or ventral striatum produced by a second injection given days to a month later, it is not known if this form of plasticity is also evident early (within 3-hours) after the first experience with cocaine. Using fast-scan cyclic voltammetry (FSCV), we sought to determine whether a single i.v. injection of cocaine leads to sensitized [DA] after the drug has been metabolized [8] and within the timeframe for the initiation of drug-induced VTA plasticity [5]. The NAc shell, and not core, was studied because drug exposure more robustly enhances DA release in the shell sub-region [9]. By investigating early plasticity following a single drug injection, we hope to determine how initial experimentation with cocaine may have long-lasting consequences, potentially increasing the chance that an individual continues to use the drug, and perhaps becomes dependent.

Male Sprague-Dawley rats weighing 250-275 g (at time of arrival; ~2 months old) were obtained from Charles River Laboratories and housed individually under a 12:12 hour reverse light/dark cycle with food and water available *ad libitum*. The experiment began 1 week after arrival and was approved by The University of Michigan Committee on Animal Use and Care.

Animals underwent separate surgeries for implantation of jugular catheters and cannula for FSCV recordings. For both surgeries, animals were anesthetized with ketamine (100 mg/kg, IM) and xylazine (10 mg/kg IM). First, chronic indwelling catheters were implanted in the right jugular vein as previously described [10]. Following recovery from catheter implantation (~ 1 week), stereotaxic surgeries were performed for insertion of a guide cannula, as well as reference and stimulating electrodes for *in vivo* FSCV. As previously described [11], a guide cannula was implanted dorsal to the NAc shell (AP, +1.8; ML, ±0.8; DV, -2.5 mm relative to

bregma; MD-2251, BASi) and an Ag/AgCl reference electrode was secured in the contralateral cortex (AP, -0.8; ML, ±4.0; DV, -2.0 mm relative to bregma). A bipolar stimulating electrode (AP, -5.2; ML, ±0.8 mm relative to bregma; C315G-MS303/2/SPC, Plastics One) was lowered into the VTA until electrically-evoked DA release was detected in the dorsal striatum, directly above the final recording location in the NAc. Surgical screws and dental acrylic were used to secure cannula and electrodes in place.

Following recovery from surgery, experiments were conducted within a Med Associates behavioral testing chamber [11]. At the beginning of the test day, saline-loaded tubing attached to a syringe pump was threaded through the commutator, along the tether, and attached to the back-mounted i.v. catheter for delivery of vehicle or drug. Next, a carbon fiber electrode (pre-calibrated using DA concentrations of 125 nM, 250 nM, and 1000 nM) was loaded into a micromanipulator (custom-manufactured) and lowered into the NAc shell and tested for its ability to detect both naturally occurring and electrically evoked DA release (via VTA stimulation; 60Hz/24-pulse, 60Hz/12-pulse, 60Hz/6-pulse, 30Hz/24-pulse, 30Hz/12-pulse, 30Hz/6-pulse, 20Hz/4-pulse). As previously described [11], FSCV relied upon the oxidation and reduction of the analyte of interest (i.e. DA) in response to the application of a triangular waveform (oxidative scan, -0.4 to 1.3 V; reductive scan, 1.3 V to -0.4 V; 10Hz, 400V/s). Background-subtracted current changes were plotted in false color (Figure 2) in order to visualize the presence of DA within the NAc shell.

FSCV recordings of “real-time” DA levels were first recorded following an i.v. saline infusion. Animals then received one cocaine HCl infusion (1.0 mg/kg, dose refers to weight of the salt), followed by a second cocaine infusion two hours later. One hour after the second cocaine injection, a subset of rats received a second i.v. saline infusion. All infusions (0.2 ml/infusion i.v.) were given over 3 seconds and FSCV measurements were recorded both immediately before and after the infusion. At the end of the experiment, VTA stimulations were repeated to ensure that the electrode remained sensitive for DA detection.

Principal component analysis was initially used for identification of DA [11; using pre and post VTA stimulations, electrode pre-calibration]. Next, DA levels were quantified 5s before and 85s after each i.v. infusion. Data were background subtracted at the time-point of lowest current before infusion. To identify individual variation in [DA] after saline or cocaine, nonparametric Wilcoxon Signed Rank Tests were carried out on each rat. In addition, mean changes in [DA] levels were analyzed using a linear mixed model procedure [9]. Furthermore, the mean [DA] area-under-the-curve following each injection was quantified and analyzed as a percent change

from baseline using a one-way ANOVA. Statistical analyses were conducted in SPSS (Chicago, IL) and GraphPad Prism (San Diego, CA).

Following testing, animals were euthanized via overdose of Beuthanasia-D (50mg/kg, IP) and then an electrolytic lesion (tungsten electrodes, 20 μA for 15 s) was made at each recording site [11]. Animals were then decapitated and brains extracted and stored in formalin. Five to seven days later, coronal brain slices (40 μm) were collected and stained with cresyl violet. Electrode placements were verified using light microscopy and referenced according to a brain atlas [Figure 1C; 26]. Only rats with electrodes located in the NAc shell were used.

The initial i.v. injection of saline (S1) had no effect on DA ($F_{84.55}$=0.56, p=0.90; linear mixed-model, 5-85s post-infusion; Fig. 1A, N=8). In contrast, both the first (C1) and second (C2) i.v. cocaine infusions significantly increased [DA] in the NAc shell (C1, $F_{89.26}$=1.89, p=0.035; C2, $F_{103.76}$=1.98, p=0.024; linear mixed-model, 5-85s post-infusion). Importantly, Fig. 1 also shows that the second cocaine infusion resulted in sensitized extracellular [DA], as indicated by a more rapid onset of the drug effect and by an analysis of the total post-infusion [DA] (85-seconds) area-under-the-curve (Figure 1B; $F_{2,14}$=14.06, **p=0.0058; C1 or C2 vs S1, p<0.01-0.05; C1 vs C2, p<0.05; one-way repeated measures ANOVA with post-hoc Bonferroni). Even though the second cocaine injection produced a greater increase in NAc shell DA levels than the first in every rat tested (within-rat comparisons, Wilcoxon Signed Rank Tests, p<0.05-0.001; C2 vs S1 or C1), some rats displayed much greater increases in [DA] than others. Accordingly, we illustrate examples from the rat that displayed the largest increase in [DA] (Figure 2A; post-injection color plots (i-iv) and calculated [DA] (v)), as well as an animal that displayed a smaller, but still significant, enhancement in [DA] following the second cocaine injection (Figure 2B; post-injection color plots (i-iv) and calculated [DA] (v)). Together, these findings suggest that the induction of sensitized DA overflow in the NAc shell can occur very rapidly, within 2-hours following the initial exposure to cocaine.

It was possible that during the first cocaine infusion rats learned to associate certain cues with cocaine delivery (e.g., sound of infusion pump, interoceptive stimuli associated with infusion; [13,14]). If this was the case, then cues associated with the second i.v. infusion could themselves potentially enhance [DA] in the NAc shell, regardless of whether cocaine or saline was given. Thus, after rats had their second cocaine injection, a subset of animals (n=5) were administered a second saline infusion. Similar to the first saline infusion (S1; see above), this second saline infusion had no effect on [DA] in the NAc shell ($F_{41.169}$=0.47, p=0.95; linear mixed-model, 5-85s post-infusion). Furthermore, there were no observed differences between the concentration of DA measured in the NAc shell following the first (S1) and last (S2) saline

injections (t_4=1.23, p=0.29; 85-seconds, area-under-the-curve). Thus, it is unlikely that conditioned DA release accounts for the enhancement in [DA] observed after the second cocaine infusion. Instead, we conclude that a single cocaine injection was sufficient to induce rapid neuroplasticity required for the development of sensitized DA overflow in the NAc shell. Finally, a potential drawback of the experimental design was that a small amount of cocaine (~0.015ml, ~7.5% of the 1mg/kg cocaine infusion; ~0.075mg/kg dose) may have remained in the catheter after the first or second drug infusion. We do not believe this impacted DA neurotransmission because the DA response following the final saline injection was indistinguishable from the initial pre-drug saline infusion.

It is well-known that a single injection of cocaine quickly initiates plasticity on DA neurons in the VTA, underlying the induction of psychostimulant sensitization. Such neuroadaptations in the VTA impact cocaine's ability to increase DA concentrations throughout the brain, with the most robust elevations often observed in the NAc shell. Until now, it has been unclear as to how rapidly this expression of sensitized DA release in the ventral striatum develops. The present results illustrate sensitization of extracellular [DA] within this region just 2-hours after an initial exposure to cocaine. At this time-point, plasma and brain cocaine levels have returned to baseline [8]. While the cocaine metabolite benzoylecgonine may still be present 2-hours after the first cocaine infusion [8], benzoylecgonine neither stimulates the formation nor the release of DA [15]. Thus, we hypothesize that sensitization-related adaptations, rather than the pharmacokinetics of cocaine, account for the enhancement in DA neurotransmission observed.

Our observation of the rapid emergence of DA sensitization is consistent with neuronal plasticity in the VTA initiated by a single cocaine injection, as LTP-like adaptations in the VTA have been previously shown to occur within 3-hours of initial drug exposure [5]. This potentiation is dependent upon both DA D5-type receptor activation of NMDARs and protein synthesis, promoting the insertion of high-conductance GluA1-containing AMPARs [5]. Therefore, these neuroadaptations, along with the present findings, may indicate facilitation of the depolarization of DA neurons induced by the second cocaine injection, potentially resulting in coordinated firing of action potentials in the VTA and sensitized [DA] in the NAc shell.

Alternatively, the first cocaine injection could have enhanced DA synthesis, thereby increasing DA content in terminals releasing the neurotransmitter [16]. This could have magnified cocaine's ability to mobilize reserve pools of DA vesicles, thereby enabling the second cocaine injection to elicit sensitized DA release. Supporting this hypothesis, sensitized DA release is observed in striatal/NAc slice preparations in the absence of VTA DA cells of

origin [17]. Accordingly, it would appear unreasonable to conclude that rapid neurochemical sensitization by cocaine occurs by a single midbrain or terminal mechanism. Rather, the process of sensitization (its induction and expression) occurs at multiple levels, demonstrating that the brain possess the ability to rapidly adapt and enhance its potential to increase extracellular [DA] in the ventral striatum.

Several lines of evidence suggest that psychostimulant-induced changes in VTA plasticity may promote long-lasting enhancements in DA synthesis. While sensitization is associated with an increase in tyrosine hydroxylase (TH) expression in the VTA, the rate-limiting enzyme of DA synthesis, this may not have occurred just 2-hours after an initial cocaine exposure [18]. Despite this, it remains conceivable that the increase in activation and insertion of NMDA and GluA1-containing AMPARs in VTA [5], as described above, may have indirectly enhanced DA synthesis. Influx of calcium via these receptors can result in the activation of calcium-calmodulin dependent kinase 2 (CaMKII; [18]). CaMKII stimulation has been shown to be necessary for the induction of sensitization to cocaine [20] and CaMKII itself can phosphorylate and activate TH [21], promoting the synthesis of DA.

While the present results demonstrate sensitization of DA levels in the NAc shell, individual rats showed marked variation in [DA] (Figure 2). Different magnitudes of DA release may reflect either individual variation in the degree of sensitization, or, less interestingly, slight differences in recording locations. Indeed, different areas of the VTA send region-specific projections to the NAc [22], and these VTA sub-regions may differentially encode information regarding rewarding and aversive events [23].

The present study focused on DA neurotransmission in the NAc shell, and not in the NAc core, because cocaine exposure produces greater enhancement of DA release in the shell sub-region [9]. Furthermore, regardless of whether cocaine is actively taken or passively administered during initial exposure, [DA] is increased more in the shell than in the core [24]. Other than altering DA signaling, cocaine administration has additional physiological (e.g., heart rate, blood pressure) and neurochemical (e.g., norepinephrine) consequences. It remains possible that these responses could indirectly contribute to the sensitization of DA release observed. However, these biological systems would have likely returned to baseline function 2-hours after the first cocaine injection [25,26].

Sensitization-related plasticity occurs very rapidly in the VTA, which is thought to be the initial site where sensitization is induced. The present results indicate that drug-induced plasticity occurs equally rapidly within the nucleus accumbens, which is thought to be critical for the behavioral expression of behavioral sensitization. Several neuroadaptations may be

responsible for the drug-induced enhancement in DA levels 2-hours after the first cocaine exposure, including (but not limited to) the initiation of LTP-like plasticity in the VTA, increased synthesis of DA, and/or mobilization of DA-containing vesicles to neuron terminals in the NAc. It is also possible that neuronal changes such as these could vary across individuals, as we observed considerable variation in the magnitude of cocaine-induced [DA] across rats.

Figure Legends

Figure 1. Sensitization of NAc [DA].

(**A**) Average changes in [DA] are shown (n=8) following the first saline injection (saline 1, S1), the first cocaine injection (cocaine 1, C1), the second cocaine injection (cocaine 2, C2), and the final saline infusion (saline 2). According to linear mixed models regressions, both the first and second cocaine injection significantly increased [DA] in the NAc shell, relative to baseline (*, $p<0.05$). (**B**) The area under the curve (based on the data shown in **A**), a measure of total DA overflow in the NAc shell, was significantly increased following both the first (**, $p<0.01$) and second (*, $p<0.05$) cocaine infusion (relative to the first saline infusion). Also, compared to the first cocaine injection, the second cocaine infusion caused a significantly greater (i.e., sensitized) increase in [DA] within the NAc shell (+, $p<0.05$). (**C**) Coronal brain sections detailing locations of FSCV recordings in the NAc shell [12]. A cresyl violet stained hemisection of a brain slice is also displayed. As can be seen and detailed in the methods, when sacrificing rats the brain was purposely lesioned at the site of previous FSCV recordings to better visualize its location. Data are shown as mean ± SEM.

Figure 2. *Individual Variation in Initial and Sensitized DA Response to Cocaine.*

Representative color plots and [DA] from 2 individual rats, (**A**) and (**B**). Data displayed from 90-second FSCV files, with the i.v. infusion of saline (**ABi,iv**) or cocaine (**ABii,iii**) occurring 5-seconds into the recording. The injection order was as follows: (**i**) “saline 1”, (**ii**) “cocaine 2,” 15-minutes after initial saline, (**iii**) “cocaine 3,” 2-hours after first cocaine injection, and finally (**iv**) “saline 2,” 1-hour after the second cocaine infusion. Color plots (**i-iv**) show current changes recorded on the carbon fiber electrode, plotted against the applied voltage (Eapp; -0.4 V to +1.3 V and back to -0.4 V, at 10Hz) and time. Calibrated [DA] traces are shown (**ABv**). ***, $p<0.001$, Wilcoxon Signed Rank Tests.

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